Acquired platelet antagonism: off-target antiplatelet effects of malignancy treatment with tyrosine kinase inhibitors

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REVIEW ARTICLE

Acquired platelet antagonism: off-target antiplatelet effects of malignancy treatment with tyrosine kinase inhibitors

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Summary. Platelets can contribute to tumor progression and metastasis. Cancer patients are at increased risk of thrombosis, and advanced stages of cancer are associated with thrombocytosis or increased platelet reactivity. Tyrosine kinase inhibitors (TKIs) are widely used as a targeted strategy for cancer treatment, with the aim of prolonging progression-free survival of the patients. Because of their broad kinase target spectrum, most TKIs inevitably have off-target effects. Platelets rely on tyrosine kinase activity for their activation. Frequently observed side effects are lowering of platelet count and inhibition of platelet functions, whether or not accompanied by an increased bleeding risk. In this review, we aim to give insights into: (i) 38 TKIs that are currently used for the treatment of different types of cancer, either on the market or in clinical trials; (ii) how distinct TKIs can inhibit activation mechanisms in platelets; and (iii) the clinical consequences of the antiplatelet effects of TKI treatment. For several TKIs, the knowledge on affinity for their targets does not align with the published effects on platelets and reported bleeding events. This review should raise awareness of the potential antiplatelet effects of several TKIs, which will be enhanced in the presence of antithrombotic drugs.

Keywords: cancer; platelets; signaling; therapy; tyrosine kinase inhibitor.

Introduction

Human blood platelets are required for hemostasis, and also contribute to pathological thrombosis [1]. Platelets normally circulate in the blood at a concentration of 150–450 $\times$ 10^9 L^{-1}, with a lifespan of 10 days. Thrombocytopenia, defined as a platelet concentration below which bleeding risks increase, is defined as a count of $< 50 \times 10^9$ L^{-1}. By implication, only a fraction of the normally circulating platelets seems to be required for proper hemostasis, as could be demonstrated in mice [2]. Hence, the majority of circulating platelets are likely to contribute to other physiological processes, which can include maintenance of vascular integrity, tissue repair, immune responses, and infection prevention. The existence of different populations of platelets, primed by their environment [3], may even suggest a certain degree of specialization in these functions. Considering the 'overload' of circulating platelets, it is not a surprise that these anucleate cells also contribute to pathological processes, including cancer.

It is well known that cancer patients are at increased risk for thrombosis [4], and that advanced stages of cancer are associated with increased platelet reactivity [5]. Recent studies have also suggested that the growth factor composition and even the RNA profile of platelets can change because of the presence of cancer [6,7]. Also, malignancy can lead to thrombocytosis (elevated platelet count), which is regarded as a negative predictor of survival [8]. The suggested mechanism is enhanced platelet formation by megakaryocytes, resulting from tumor-derived interleukin-6 production and high thrombopoietin production in the liver [9]. This points to a cycle of tumor-induced platelet formation and activation, followed by growth factor release and tumor promotion and metastasis. This reciprocal interaction between platelet activation and tumor growth has recently been reviewed by others [10]. The model proposed in 2000 is that tumors secrete chemokines that recruit blood cells, including platelets, and thus promote the process of angiogenesis, i.e. the formation of new blood vessels from pre-existing vessels, in order to secure tumor development and metastasis [11]. Platelets contain numerous proangiogenic (as well as antiangiogenic) molecules in their α-granules, which are either synthesized by megakaryocytes in the bone marrow or are taken up via endocytosis [12].

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In the majority of cancers, oncogene-encoded proteins or growth factor receptors are amplified or mutated, which either carry intrinsic tyrosine protein kinase activity or control both tyrosine and serine/threonine kinases in the downstream signaling pathways. Accordingly, abnormal protein kinase activity is considered to be a hallmark of tumor biology [13], resulting in altered cell proliferation, survival, motility, metabolism, and angiogenesis, as well as evasion of immune responses [14]. This insight has prompted the search for tyrosine kinase inhibitors (TKIs) as targeted therapeutic drugs in oncology.

Protein tyrosine kinases are classified into the receptor-linked tyrosine kinases, e.g. the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors, and the cytosolic non-receptor kinases, such as Src family kinases (SFKs), Syk, and Btk. In tumors, gain-of-function mutations are often seen in the genes encoding tyrosine kinases, resulting in constitutively active proteins. Examples of such aberrant oncoproteins are BCR–ABL and NPM–ALK. These are encoded by fusion genes that originate from reciprocal translocation of genetic material between two chromosomes, resulting in proteins that are 'always on', and leading to uncontrolled cell division. In the past 15 years, almost 40 TKIs have been developed and approved for cancer treatment (Table 1). In most cases, these are small molecules with relatively high affinity for the so-called targeted kinases, but often with similar affinities for other tyrosine kinases. As many of these tyrosine kinases are also expressed in non-proliferative cells, including platelets, TKIs can clearly have relevant off-target effects.

Blood platelets are unable to proliferate or differentiate, but show high tyrosine kinase activities in comparison with other cell types. In platelet activation, tyrosine phosphorylation is one of the key signal transduction mechanisms. As described below, non-receptor tyrosine kinases are activated by the collagen receptor glycoprotein (GP) VI, C-type lectin-like receptor 2 (CLEC-2), von Willebrand factor (VWF) receptor, GPIb–IX–V, and adhesion-regulating integrins [15,16]. In this review, we aim to give insights into: (i) the TKIs that are currently used for the treatment of different types of cancer, either on the market or in clinical trials; (ii) how distinct TKIs can inhibit activation mechanisms in platelets; and (iii) the clinical consequences of the antiplatelet effects of TKI treatment.

Cancer treatment with TKIs

TKIs are defined as pharmaceutical drugs that inhibit tyrosine kinases. The majority of TKIs that are currently in use are small molecules that can cross the cell membrane. Their common mode of action is competition with ATP in the conserved catalytic binding site in the superfamily of (non)-receptor tyrosine kinases. Because of the comparable structures of the catalytic pockets of tyrosine kinases, TKIs often target multiple kinases that play roles in several signaling pathways. At present, 40 drugs with TKI activity have been approved by the USA Food and Drug Administration, or have entered clinical trials for anticancer treatment (Table 1).

On the basis of their precise action, TKIs can be classified into four categories [17]. Type I inhibitors target the active conformation of the kinase, and compete with the ATP-binding site (e.g. sunitinib). Type II inhibitors instead recognize the inactive conformation, and thereby indirectly compete with ATP by occupying a hydrophobic pocket near the ATP-binding site. Accordingly, type II inhibitors are considered to modify the kinase activity in an allosteric manner (e.g. imatinib and sorafenib). Type III inhibitors are the so-called allosteric inhibitors. These bind more distantly from the ATP-binding site, and inhibit kinase activity via classical allosteric interference (e.g. MEK1 inhibitors such as selumetinib). Type IV inhibitors are also known as covalent inhibitors, forming an irreversible covalent bond near the active site of the kinase, usually by reacting with a cysteine. This blocks the binding of ATP and prevents activation of the kinase (e.g. ibrutinib and vantedanib).

Although they are directed against specific targets, almost all TKIs affect multiple kinases. Table 1 provides an overview of the currently used TKIs that are prescribed for different types of cancer, together with their target kinases in tumor cells. The majority of the drugs are targeted at receptor tyrosine kinases for growth factors (epidermal growth factor [EGF], fibroblast growth factor, PDFG, and VEGF) or for differentiation/proliferation factors (FLT, FMS, Kit, and Ret). Several of the drugs also target intracellular tyrosine kinases (ABL, B-Raf, Btk, Itk, MEK, SFKs, and Syk). Although affinities for individual kinases will differ, essentially all TKIs have more or less broader efficacy, which in some cases is intentional. For instance, both VEGF and PDGF are known to be important in tumor angiogenesis. Sunitinib, which blocks signaling via both types of receptor, will thus have a broader spectrum of action spectrum vatalanib, which targets only VEGF receptors [18]. In general, the use of TKIs for the treatment of specific tumors depends on the affinity for the targeted kinases [19] and the pharmacokinetics at the indicated dosages and with treatment regimens [20] (Table 1). As far as is understood, the ultimate specificity is not strongly correlated with chemical structure or the subfamily of the targeted kinase [18].

Receptor-linked tyrosine kinases are also abundantly expressed in non-tumor cells, and treatment with TKIs will inevitably have off-target effects, thus interfering with the normal function of non-diseased cells and tissues. This can explain the side effects of treatment, varying from general complications such as fatigue, diarrhea, and nausea, to specific complications such as hand–foot syndrome [21]. In spite of drug-to-drug variations, many TKIs can cause skin toxicity, even in > 50% of patients

Table 1 Overview of tyrosine kinase inhibitors (TKIs) prescribed to cancer patients or evaluated in clinical trials

TKI*	Commercial name	Type of TKI	Reported targets	Used for treatment of:	Line treatment†	Dosage (treatment regimen)†	Bleeding/ thrombosis reported	Reference
Acalabrutinib	Calquence	IV	Btk, Tec	CLL, MCL, NHL	1 or 2	100–400 mg once daily 100 mg twice daily	Bleeding	[45]
Afatinib	Gilotrif, Giotrif	I	EGFR, ErbB2–4	NSCLC	1	20-50mg once daily, continuous daily dosing	Bleeding	[64,65]
Apatinib (phase III)	YN968D1	ND	VEGFR2	BC, GC, HCC, NSCLC, PC	NA	750 mg once daily, continuous daily dosing		[83]
Axitinib	Inlyta	II	VEGFR1–3	RCC	1	5 mg twice daily, continuous daily dosing	Bleeding	[20,63]
Bosutinib	Bosulif	I	Abl, Src	CML	1	500 mg once daily, continuous daily dosing	Bleeding	[59,84]
Brivanib	BMS-582664		FGFR1, VEGFR	HCC, MCRC, solid tumors	1	800 mg once daily, continuous daily dosing		[85]
Cabozantinib	Cabometyx, Cometriq	II	Axl, Flt3, Kit, MET, Ret, VEGFR2	TC RCC	1 2	140 mg once daily, continuous daily dosing 60 mg once daily, continuous daily dosing	Bleeding	[63,86]
Cediranib	Recentin	ND	Kit, PDGFR, VEGFR1–3	HCC, RCC	1 or 2	30–45 mg once daily, continuous daily dosing		[87]
Ceritinib	Zykadia	I	Alk	ALK^+ NSCLC	1	750 mg once daily, continuous daily dosing		[88]
Cobimetinib	Cotellic	ND	MEK1–2	Melanoma	1	60 mg (once daily, 3/1)		[89]
Crizotinib	Xalkori	I	Alk, MET, ROS1	NSCLC	1	250 mg twice daily, continuous daily dosing		[20,90]
Dabrafenib	Tafinlar	I	B-Raf	Melanoma, NSCLC, TC	1	150 mg twice daily, continuous daily dosing		[91]
Dacomitinib (phase III)	PF-00299804	ND	EGFR, ErbB1–2, ErbB4	NSCLC	NA	45 mg once daily, continuous daily dosing		[92]
Dasatinib	Sprycel	I	BCR-ABL, EphA2, Kit, PDGFR, SFK	CP CML AP MB, LB CML	2	100-140 mg once daily, continuous daily dosing 70 mg twice daily, continuous daily dosing	Bleeding	[20,93]
Dovitinib	TKI258	ND	CMS1, FGFR1-3, Flt3, Kit, PDGFR, VEGFR1-3	GIST, melanoma, RCC	2 or 3	500 mg once daily, 5/2		[94]
Entospletinib (phase II)	GS-9973	ND	Syk	CLL	NA	800 mg twice daily, continuous daily dosing		[95]
Erlotinib	Tarceva	I	EGFR	NSCLC PC	2 or 3	100–150 mg once daily, continuous daily dosing		[20,96]
Fostamatinib (phase II)	R788	ND	Syk	B-cell lymphoma, CLL	NA	200 mg twice daily, continuous daily dosing		[97]
Gefitinib	Iressa	I	EGFR	NSCLC	1	250 mg once daily, continuous daily dosing		[20,98]
Ibrutinib	Imbruvica	IV	Btk, Tec	CLL, MCL, NHL	1 or 2	420 mg once daily, continuous daily dosing	Bleeding	[34,37,38]
Icotinib (phase III)	BPI-2009H	ND	EGFR	Brain cancer, NSCLC	NA	125 mg three times daily, continuous daily dosing		[98]

Table 1 (Continued)

TKI*	Commercial name	Type of TKI	Reported targets	Used for treatment of:	Line treatment†	Dosage (treatment regimen)†	Bleeding/ thrombosis reported	Reference
Imatinib	Gleevec, Glivec	II	BCR-ABL, DDR, Kit, PDGFR	ALL, AP CML, CP CML, KIT^+ GIST	1	400–600 mg once daily, continuous daily dosing	Bleeding	[20,99]
Ipatasertib (phase II)	RG7440	ND	Akt	BC, CRC, NSCLC, OC, PRC	NA	400 mg once daily, 3/1		[100]
Lapatinib	Tykerb	II	EGFR, ErbB1-2	$ErbB2^+$ BC $ErbB2^+$ and HR^- BC $ErbB2^+$ and HR^+ BC	1 or 2	1000–1500 mg once daily, continuous daily dosing		[20,101]
Lenvatinib	Lenvima	II	FGFR, Kit, PDGFRα, Ret, VEGFR	RCC, TC		24 mg once daily, continuous daily dosing		[63,102]
Nilotinib	Tasigna	II	BCR-ABL, CSF-1R, DDR, Kit, PDGFR	CP CML, GIST	1	300–400 mg twice daily, continuous daily dosing	Thrombosis	[20,99]
Nintedanib	Ofev, Vargatef	II	FGFR, FMS, PDGFR, VEGFR	IPF, advanced NSCLC	1 2	150 mg twice daily, continuous daily dosing	Bleeding	[63,103]
Osimertinib	Tagrisso, Tagrix	ND	EGFR	NSCLC	2	80 mg once daily, continuous daily dosing		[104]
Pazopanib	Votrient	II	FGFR, FMS, Itk, Kit, Lek, PDGFR, VEGFR	NSCLC, OC, RCC, STS, TC	1	800 mg once daily, continuous daily dosing	Bleeding	[20,63]
Ponatinib	Iclusig	II	BCR-ABL, FGFR, KIT, PDGFR, Ret, Src, VEGFR	Ph^+ ALL, CML	1 or 2	30 mg once daily, continuous daily dosing	Bleeding	[105]
Regorafinib	Stivarga	II	B-Raf, FGFR, Kit, PDGFR, Ret, Tie2, VEGFR1–3	Advanced GIST, MCRC	3	160 mg once daily, 3/1		[106]
Selumetinib	AZD6244	III	MEK1–2	KRA-mutated NSCLC	2 or 3	75 mg twice daily, continuous daily dosing		[107]
Sorafenib	Nexavar	I	VEGFR	HCC, RCC	1–3	400 mg twice daily, continuous daily dosing	Bleeding	[20,60, 63,85]
Sunitinib	Sutent	I	CSF-R, Flt3, Kit, PDGFR, VEGFR	RCC, GIST, PNET	1 or 2	37.5–50 mg once daily, 4/2	Bleeding	[20,60, 61,63]
Trametinib	Mekinist	III	MEK1–2	BRAF $V600^+$ melanoma	1	2 mg once daily, continuous daily dosing	Bleeding	[108]
Vandetanib	Caprelsa	IV	EGFR, Ret, VEGFR	MTC, NSCLC	1 or 2	300 mg once daily, continuous daily dosing		[20,109]
Vatalanib (phase III)	PTK787/ ZK-222584	ND	Kit, PDGFRβ, VEGFR	MCRC	1	1.25 mg once daily, continuous daily dosing		[110]
Vemurafenib	Zelboraf	I	B-Raf	BRAF $V600^+$ melanoma	2 or 3	960 mg twice daily, continuous daily dosing		[20,111]

ALK, anaplastic lymphoma kinase; ALL, acute lymphocytic leukemia, AP, acute-phase; BC, breast cancer; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CP, chronic-phase; CRC, colorectal cancer; CSF-R, colony-stimulating factor receptor; CSF-1R, colony-stimulating factor 1 receptor; EGFR, epidermal growth factor receptor; ErbB2, human epithelial growth factor 2; FGFR, fibroblast growth factor receptor; GC, gastric cancer; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HR, hormone receptor; IPF, idiopathic pulmonary fibrosis; LB, lymphoid blast; MB, myeloid blast; MCL, mantle cell lymphoma; MCRC, metastatic colorectal cancer; MTC, medullary thyroid cancer; NA, not applicable; ND, not determined; NHL, Non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; OC, ovarian cancer; PC, pancreatic cancer; PDGFR, platelet-derived growth factor receptor; Ph+, Philadelphia-positive; PNET, primitive neuroectodermal tumor; PRC, prostate cancer; RA, rheumatoid arthritis; RCC, renal cell carcinoma; SFK, Src family kinase; STS, soft tissue sarcoma; TC, thyroid cancer; VEGFR, vascular endothelial growth factor. *Small-molecule inhibitors of kinase enzymes are indicated with the suffix 'nib'. TKIs are indicated with the suffix 'tinib'. Angiogenesis inhibitors are indicated with the suffix 'anib'. Rapidly accelerated fibrosarcoma (RAF) kinase inhibitors are indicated with the suffix 'rafenib'. †3/1, 3 weeks of dosing, followed by 1 week of rest; 4/2, 4 weeks of dosing, followed by 2 weeks of rest; 5/2, 5 days of dosing, followed by 2 days of rest.

[22]. For antiangiogenic TKIs, 'blood-related' side effects have been reported, such as hypertension, myelosuppression, and bleeding [23]. Incidentally, on-treatment cardiovascular events have also been reported, e.g. linked to affected vascular integrity of the endothelium [24]. TKI effects on the vessel wall comprise endothelial dysfunction and increased capillary leakage [25], which may contribute to an increased bleeding tendency. Paradoxically, drugs affecting the integrity of endothelial cells can shift the hemostatic balance in favor of thrombosis; this may explain why treatment with specific TKIs has been associated with an increase in arterial thromboembolic events in cancer patients [25,26].

Despite the clinical benefits of the prescribed TKIs, some of the patients who initially respond to the therapy experience a relapse, e.g. because of acquired drug resistance of the tumor [14]. In such cases, the treatment schedule is adjusted or a switch is made to an alternative TKI as a second-line or third-line treatment (Table 1). In specific cases, several TKIs can be combined for effective blockade of one or two signaling pathways [14,21]. Furthermore, chemotherapy or radiation therapy can be complemented with TKI treatment [13].

Protein tyrosine kinases implicated in platelet activation

A global overview of platelet tyrosine kinase signaling underneath several receptors, as well as the downstream platelet responses, is given in Table 2.

GPVI signaling Collagen-induced platelet activation is established via the tyrosine kinase-linked receptor GPVI, which is a member of the immunoglobulin superfamily. GPVI is linked to the Fc receptor γ chain (FcRγ), which contains two immunoreceptor tyrosine-based activation motifs (ITAMs) that require phosphorylation to mediate platelet activation [27]. Ligand binding and dimerization of the GPVI–FcRγ complex leads to activation of the SFK isoforms Src, Fyn, and Lyn, which, in turn, phosphorylate the FcRγ ITAM tyrosine residues to recruit and phosphorylate Syk [1,28]. The tyrosine kinases SFK and Syk also phosphorylate downstream targets, including the transmembrane adapter linker for activated T cells (LAT) and Src homology 2 domain-containing leukocyte phosphoprotein (SLP76). The consequence is the formation of a large signaling complex, including LAT, SLP76, Btk, isoforms of phosphoinositide 3-kinase (PI3K), and Tec family kinases [29]. A key downstream event is the phosphorylation and activation of the second messenger-generating phospholipase (PLC) γ2, resulting in Ca^{2+} mobilization and protein kinase C activity. Further responses are integrin activation, thromboxane A_2 release, granule secretion, and phosphatidylserine exposure.

Table 2 Protein tyrosine kinases implicated in platelet activation responses: global overview of tyrosine kinases implicated in signaling via key platelet receptors, as well as downstream platelet responses. Summarized from [1,16,28,30]

	Signaling tyrosine kinases		
Receptor	SFK	Other	Platelet response
GPVI	Src, Fyn, Lyn, Fgr	Btk, Syk, Tec	Ca^{2+} mobilization, integrin activation, degranulation
CLEC-2	Src, Fyn, Lyn	Btk, Syk, Tec	Ca^{2+} mobilization, integrin activation, degranulation
GPIb–IX–V	Src, Fyn, Lyn	Btk	Integrin activation
Integrin $\alpha_{IIb}\beta_3$	Src, Fyn, Lyn	FAK, Syk, SLP76, Pyk2	Spreading, outside-in signaling, clot retraction

CLEC-2, C-type lectin-like receptor 2; GP, glycoprotein; SFK, Src family kinase; SLP76, Src homology 2 domain-containing leukocyte phosphoprotein.

CLEC-2 signaling A similar powerful activation pathway of platelets is induced via CLEC-2, a C-type lectin receptor that also acts through tyrosine phosphorylation. Known ligands of this receptor are podoplanin (expressed in tumor tissue, among other tissues) and the snake venom rhodocytin. Signaling occurs through a so-called hem-ITAM motif [30]. The clustering of CLEC-2 induces events that are more or less similar to those described for GPVI. Starting with the tyrosine phosphorylation of SFK and Syk, a signaling complex including Tec family tyrosine kinases is formed, resulting in PLCγ2 and PI3K activation. The consequences are, again, integrin activation, granule release, and thromboxane A_2 production [30].

GPIb–IX–V signaling Interaction of VWF with the GPIb–IX–V receptor is one of the first steps in platelet tethering and adhesion under shear flow [1]. This interaction causes only weak signaling, e.g. leading to restructuring of the actin cytoskeleton, with, under certain conditions, phosphorylation of SFKs (Src, Fyn, and Lyn) and activation of PI3K isoforms [1]. Integrin $\alpha_{IIb}\beta_3$ activation and platelet spreading result from this.

Integrin-dependent signaling Platelet integrins, in particular $\alpha_{IIb}\beta_3$, $\alpha_2\beta_1$, and $\alpha_v\beta_3$, regulate adhesion, aggregation, and thrombus formation [28]. Especially regarding integrin $\alpha_{IIb}\beta_3$ (ligands: fibrinogen, VWF, and other matrix proteins) much research has been performed on the outside-in signaling events triggered by the occupied, activated conformation. Several tyrosine kinases are implicated in this signaling pathway, including FAK, Pyk2, Src, SLP76, and Syk [29,31].

Effects of TKIs on platelet function

Tyrosine kinases are targeted by TKIs as a treatment for cancer, their most important effect being the prolongation

of progression-free survival. Given the presence of on-target or related off-target tyrosine kinases in megakaryocytes and platelets, it is to be expected that several TKIs will interfere with platelet formation and/or platelet activation processes.

To provide an overview, we evaluated the inhibition profiles of TKIs (22 in total) against target protein tyrosine kinases that are known to be expressed in platelets [32]. Notably, the majority of these TKIs showed relatively low affinities for kinases with crucial roles in platelet activation processes (SFKs, Syk, Btk, MEK, and Eph), exceptions being bosutinib, dasatinib, fostamatinib, nintedanib, and sunitinib (Fig. 1). For such an *in vitro* affinity-based analysis [18,19,33], it should be realized that the presence of blood plasma and blood cells can profoundly change the bioavailability of a TKI, apart from its metabolic and pharmacokinetic profile. Notably, bleeding symptoms have been reported for 11 of these TKIs. For individual TKIs, on the basis of their known targets, we have summarized in Table 3 which of these targets are expressed in platelets. Furthermore, we made an inventory of the published effects of these TKIs on platelet responses.

Ibrutinib is a covalently acting TKI that is targeted at Btk and Tec, which probably explains the strong effects observed on platelets [34]. It is frequently used for the treatment of mantle cell lymphoma and chronic lymphocytic leukemia (CLL). Emerging data suggest that ibrutinib could also be used to treat solid tumors [35], and it has been shown to inhibit several other kinases, such as Itk, JAK3, Hck, Blk, EGF receptor (EGFR), ErbB2, and ErbB4 [36]. Ibrutinib treatment is associated with a risk of bleeding [34,37,38]. Being the most investigated TKI with regard to platelets, ibrutinib has been shown to potently inhibit

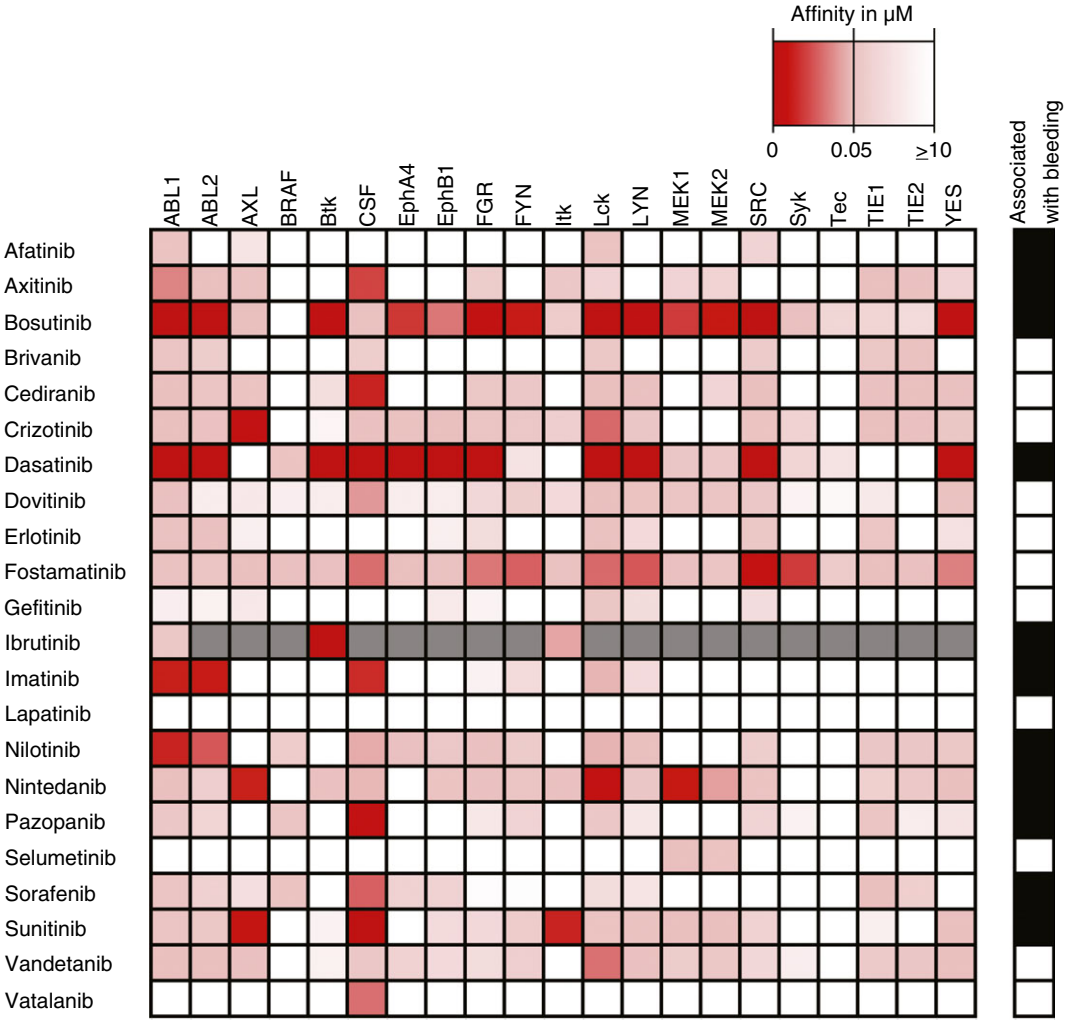

Fig. 1. Heatmap of affinity profile of tyrosine kinase inhibitors (TKIs) with described targets that are known to be expressed in platelets. Shown are normalized affinity-based dissociation constants (K_d) of the indicated TKIs determined for purified kinases [18,19,33,36], as far as they are present in platelets. Scaling is from 0 to 10, with the lowest K_d indicating the highest affinity (deep red is highest affinity = lowest Kd; white is lowest affinity = highest Kd; pink gradient represents intermediate Kd's; gray values have not been described). The right column indicates increased bleeding risk (black, yes; white, no or unknown); see Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

Table 3 Tyrosine kinase inhibitors (TKIs) targeting platelet activation processes

		Effect on platelets													
TKI	Targets in platelets (MKs)	Platelet count	Integrin activation	Secretion	Procoagulant activity	Spreading	Aggregation	Adhesion	Thrombus formation	Tyrosine phosphorylation	Elevated $[Ca^{2+}]_i$	Clot retraction	PFA-100 closure times	Agonist pathway	Ref
Acalabrutinib	Btk, Tec		↓	↓			↓		↔		↓			GPVI	[46]
Afatinib	Akt		↓	↓	↓		↓				↓			GPVI	[65]
Axitinib	*n.d.*	↓												ND	[63]
Bosutinib	Src						↔							ND	[52]
								↓						ND	[53]
Cabozantinib	Axl, Tie2 (Kit)													ND	
Canertinib	ErbB													ND	
Cediranib	(Kit)													ND	
Cobimetinib	MEK1-2													ND	
Dabrafenib	B-Raf													ND	
Dasatinib	Src (Kit)			↓			↓	↓	↓	↓				GPVI	[51]
		↓					↓						↑	GPVI	[49]
							↓							AA; epinephrine	[52]
							↓	↓	↓					GPVI	[56]
Dovitinib	CMS1 (Kit)													ND	
Entospletinib	Syk													ND	
Fostamatinib	Syk													ND	
Ibrutinib	Btk, Tec, Src						↓							GPVI	[39]
							↓							GPVI	[41]
		↑		↓			↓						↑	GPVI	[43]
			↓		↓	↓	↓	↔	↓		↓	↓		GPVI	[40]
							↓	↓		↓				GPVI; VWF	[42]
				↓		↓	↓		↓	↓				collagen; fibrinogen	[44]
Imatinib	Lyn, Btk (Kit)			↔	↔		↔		↓	↔				GPVI	[57]
							↓		↔					GPVI	[56]
Ipatasertib	Akt													ND	
Nilotinib	Lyn, Btk			↔	↔		↔			↔				GPVI	[57]
	(Kit, Ret)		↔	↔			↔	↑	↑					GPVI	[56]
Nintedanib	FMS													ND	
Pazopanib	Itk, Lck (Kit)	↓												ND	[63]
Ponatinib	Src, Syk, Lyn, Btk (Kit)		↓	↓	↓	↓	↓	↓	↓	↓				collagen; fibrinogen	[57]
							↓						↑	GPVI	[58]
Regorafinib	Tie2, B-Raf (Kit)													ND	
Selumetinib	MEK1-2													ND	
Sorafenib	B-Raf (Kit, Ret)	↓												ND	[63]
Sunitinib	(Kit, Ret)	↓												ND	[62]
		↓			↔		↓	↓	↓	↓				GPVI	[61]
		↓												ND	[63]
Trametinib	MEK1-2													ND	
Vemurafenib	MEK1-2													ND	

AA, Arachidonic Acid; [Ca2+]i, intracellular calcium concentration; GP, glycoprotein; ND, not determined; VWF, von Willebrand factor. Listed are TKIs known to target tyrosine kinase expressed in platelets and megakaryocytes (MKs), and described activation responses and assays, and agonist pathways. Arrows: ↓ decreased; ↑ increased; ↔ not affected. ND: not determined.

collagen-induced responses of platelets from patients receiving treatment [39–44]. Efficient inhibition by ibrutinib of the GPVI pathway (in response to collagen or collagen-related peptide) has been demonstrated to proceed via reduced PLCγ2 phosphorylation [42]. In treated patients, the suppression of collagen-induced platelet aggregation has been shown to correlate with the occurrence of bleeding events [42]. Subsequent studies showed that ibrutinib suppressed multiple (mostly) GPVI-dependent platelet responses, including adhesion, spreading, calcium fluxes, secretion, phosphatidylserine exposure, and clot retraction [40,43]. In addition, evidence was obtained for reduced $\alpha_{IIb}\beta_3$-dependent outside-in signaling, linked to thrombus instability *in vitro* [40]. Several studies have confirmed that Btk can act as a central target of ibrutinib in GPVI-stimulated platelets, although downstream tyrosine kinases may be affected as well [40,42,44]. *In vitro*, ibrutinib was found to fully inhibit the tyrosine phosphorylation of Src and PLCγ2 [42]. Other affected events were the phosphorylation of Fyn, Lyn, Btk, Tec, and Syk [40,44]. Together, these findings point to potent suppression by ibrutinib of the GPVI signalosome, with the Tec family kinases as the major targets.

The second-generation drug acalabrutinib is a more selective and irreversible Btk inhibitor. In a clinical trial with patients with relapsed CLL, acalabrutinib showed promising safety and efficacy profiles, although some minor bleeds were still reported [45]. A recent study comparing treatment with ibrutinib and treatment with acalabrutinib indicated that both compounds impaired platelet aggregation responses after collagen receptor stimulation [46]. Both drugs inhibited platelet Btk and Tec at physiological concentrations, whereas only ibrutinib inhibited SFK isoforms. This provided an explanation for why only ibrutinib, and not acalabrutinib, caused dysfunctional thrombus formation [46].

Imatinib, which targets the oncogenic kinase BCR–ABL, was the first TKI developed for chronic myeloid leukemia (CML). Imatinib is known to block Kit and PDGF receptors, as well as Erk and Akt isoforms [47]. This TKI has been reported to cause thrombocytopenia in 18% of the treated patients, with hemorrhages also being reported [48]. Despite the high efficacy of imatinib, 15% of patients appear to develop clinical resistance and relapse after initial response to therapy.

Dasatinib and nilotinib have been developed as second-generation BCR–ABL inhibitors for the treatment of CML. Two case reports showed that dasatinib could induce a bleeding diathesis correlated with platelet dysfunction, which was reversed upon cessation of dasatinib treatment [49,50]. Studies with blood from patients or healthy subjects showed impairment of platelet aggregation by dasatinib given *in vivo* or *in vitro* [51,52]. In particular, platelet aggregation in response to collagen, arachidonic acid and epinephrine was affected [52], along with collagen-induced thrombus formation [51,53], suggesting interference with GPVI signaling and other pathways. The mechanism of dasatinib-induced inhibition in platelets was elucidated from changes in the protein phosphorylation profile after collagen stimulation; dasatinib appeared to interfere with SFK, PLC and PI3K activities [51]. Thrombocytopenia is a relatively common side effect of dasatinib treatment [54]. This has been proposed to be attributable to reduced megakaryocytopoiesis, resulting in lower platelet production [55]. The combined quantitative (via megakaryocytes) and qualitative (via signaling) platelet dysfunction is most likely the reason for the relatively high bleeding incidence in dasatinib-treated patients. Imatinib induces less antiplatelet activity and causes less bleeding at clinically achievable doses than dasatinib, probably because it is less inhibitory for SFK isoforms. On the other hand, nilotinib has been reported to potentiate thrombus formation and increase the risk of thrombosis [56].

Ponatinib and bosutinib are third-generation BRC–ABL inhibitors used for the treatment of CML, and are applicable when patients develop resistance to imatinib. The use of ponatinib has been associated with bleeding diatheses, along with changes in platelet count and function, which may explain the impaired hemostasis [57,58]. One study described inhibitory effects of ponatinib, imatinib and nilotinib on platelet activation, secretion and aggregation in response to collagen [57]. In this study, ponatinib appeared to be the most potent platelet inhibitor. Ponatinib increased PFA-100 closure times, probably by suppressing ITAM signaling via several tyrosine kinases, including Src, Lyn, Syk, and Btk [57,58]. In contrast, imatinib and nilotinib did not affect platelet granule secretion, procoagulant activity, or aggregation, and only moderately reduced the tyrosine phosphorylation of Lyn and Btk as compared with ponatinib [57]. However, whole blood thrombus formation was reduced by imatinib and ponatinib to similar extents. For bosutinib (which also targets Src), bleeding has also been reported [59], although in patients it did not affect platelet aggregation in response to several agonists [52]. In microfluidic whole blood testing of platelet thrombus formation, bosutinib was found to be less potent than dasatinib [53]. Overall, it appears that the BCR–ABL inhibitors used for leukemia treatment have an inhibitory effect on platelet functions, with the possible exception of nilotinib.

Sunitinib, sorafenib and pazopanib are multitarget TKIs used for the treatment of renal cell carcinoma, with the aim of inhibiting tumor angiogenesis via interference with the receptors for VEGF and PDGF. Such multitarget TKIs may potentially also inhibit tyrosine kinases present in platelets. For sunitinib and sorafenib, a meta-analysis of 23 trials (> 6500 patients) showed an increased incidence of bleeding events: 16.7% for all grades of bleeding, and a 2.4% probability of high-grade bleeding events [60]. For sunitinib, both quantitative and qualitative effects on platelets have been reported. The

compound can be taken up by platelets from healthy donors and renal cell carcinoma patients, and hence reduce platelet functionality in a way that depends on protein tyrosine phosphorylation [61]. In patients receiving treatment, this is accompanied by a decrease in platelet count [61,62]. For sorafenib, similarly to other TKIs used for the treatment of renal cell carcinoma, such as pazopanib, an increased risk of bleeding has also been observed [63], but effects on platelet function have not been investigated.

Afatinib is used for the first-line treatment of, particularly, patients with non-small-cell lung cancers [64]. It acts as an irreversible inhibitor of EGFR and human epidermal growth factor receptor. An increased bleeding risk in patients receiving afatinib has been linked to impaired platelet function and apoptosis [65]. In particular, afatinib impaired GPVI-induced platelet responses, including calcium fluxes, integrin activation, secretion, procoagulant activity, and platelet aggregation (Table 3). On the other hand, thrombin-induced platelet responses were less strongly inhibited. Further investigation showed that the collagen-induced phosphorylation of Akt was decreased by afatinib [65].

Cabozantinib, cediranib, dovitinib, lenvatinib, regorafinib and other TKIs cotarget the receptor kinase Kit, which acts as a receptor for stem cell factor in megakaryocytopoiesis, regulating platelet production. The Kit receptor is known to signal via the SFK, PI3K, Ras–MEK and JAK–STAT pathways [66]. In an analogous way, the thrombopoietin receptor Mpl signals via JAK2 and Tyk2 as immediate effector kinases, resulting in the activation of STAT isoforms [67]. For these signaling pathways, key tyrosine kinases are also present in platelets and/or megakaryocytes (Tables 2 and 3), which raises the possibility that Kit-targeted TKIs can modulate platelet function as well as platelet count. So far, the signaling effects of these TKIs on platelets have not been investigated in detail. Cabozantinib treatment has been associated with a higher bleeding risk [63]. Related drugs are ruxolitinib, pacritinib, and lesaurtinib, which also target JAK1/2 and are usually prescribed for myeloproliferative disorders, such as essential thrombocythemia, which is characterized by an elevated platelet count (thrombocytosis). The latter compounds appear to lower (normalize) the concentration of circulating platelets [68], most likely via inhibition of the thrombopoietin pathway in megakaryocytes. However, the effects on platelet function are still unclear. It is known that thrombopoietin potentiates agonist-induced platelet activation via JAK2 and Tyk2 signaling [69]. This indicates that this pathway is not strong enough to induce platelet activation on its own. Inhibition of JAK2 in platelets has been reported to only attenuate collagen-induced responses [70].

For multiple prescribed TKIs with a broad target range, bleeding symptoms have not been reported (Table 1). Examples of these are fostamatinib, dovitinib, and vantedanib, which, on the basis of their affinity profiles, should affect SFK and/or MEK isoforms in platelets (Fig. 1). In these cases, additional research is needed to verify the effects on platelet count and activation, and to determine whether there is a bleeding risk. Summarizing the current knowledge of TKI effects on platelet activation responses, it appears that the majority of multitarget TKIs suppress, in particular, GPVI-induced signaling pathways, but that they can also target kinase events downstream of other platelet receptors. As the inhibition of GPVI alone has been shown to prevent occlusive thrombus formation without causing bleeding [71], it is most likely that the targeting of pathways downstream of additional receptors is responsible for an increased bleeding risk with these compounds.

Clinical implications of platelet inhibition with TKIs

Platelets are known to interact with cancer cells in the blood. Tumors exposed to the bloodstream can activate platelets, and platelets can stimulate tumor angiogenesis, growth, and metastasis. The question has been raised of whether and how interfering in the platelet–cancer loop via antiplatelet treatment may also influence cancer progression. Several studies have reported that aspirin and $P2Y_{12}$ receptor inhibitors suppress tumor angiogenesis *in vitro* [72] and tumor growth *in vivo* [73]. A low daily aspirin intake has been associated with a reduced risk for the development of cancer, as well as the prevention of metastases [74]. The mechanism is not completely resolved, but it has been shown that inhibition of platelets by aspirin decreased their ability to stimulate cancer cell proliferation through modulation of the Myc oncoprotein [75]. For other antiplatelet drugs, clinical evidence of a beneficial effect on cancer is still lacking.

Of growing interest is the recent finding that platelets are capable of sequestering bioactive compounds from the plasma, including growth factors [12,76,77] and RNA species [7]. In addition, platelets accumulate the anticancer drugs bevacizumab [78] and sunitinib (in granules) [61]. Other TKIs can also be taken up by platelets, which usually is a requirement for interaction with the intracellular kinases. Whether these TKIs can also be secondarily released by platelets and, perhaps, then contribute to tumor inhibition is matter of speculation. Another consideration is that uptake of TKIs by platelets (e.g. in patients with thrombocytosis) can reduce the availability of the compounds for tumor inhibition. On the other hand, it is likely that a decreased aggregation tendency of platelets (as induced by TKIs) also lowers tumor-induced platelet activation, and thereby reduces metastasis of tumor cells. However, the evidence for such a mechanism is, at best indirect, and further investigation is required. Taken these findings together, it appears that platelets can interfere with drug effects in different ways and by

different mechanisms, which should be taken into account in therapeutic drug monitoring.

Application of the TKIs axitinib, dasatinib, pazopanib, sorafenib and sunitinib – all of which may cause bleeding – can lead to a reduction in the platelet concentration in blood (Table 3). In cancer patients, thrombocytosis has been associated with a worse overall prognosis than that in patients with a normal platelet count [8,9]. In addition, in glioblastoma patients who underwent concomitant radiotherapy and chemotherapy, a decrease in platelet count correlated with longer overall survival [79]. In renal cell carcinoma patients with metastasis, a reduction in platelet count after the start of sunitinib treatment was associated with a better treatment outcome [62]. Although more research is needed, these studies suggest that platelet count can be used as one of the parameters to monitor anticancer treatment efficacy.

Besides platelet count, platelet function can also be affected by many TKIs, as indicated in Table 3. This suggests that combined platelet count and function tests could be used in clinical practice to assess the risk of bleeding upon TKI treatment. For the availability of such (point-of-care) tests, we refer to the literature [80]. In the future, it may be possible to use flow-based assays for this purpose. Our laboratory recently found that this method detects additive effects of low platelet count and impaired platelet functionality in patients with a bleeding phenotype [81].

It is known that cancer patients are at increased risk for thrombosis, also referred to as Trousseau's syndrome [82]. As a consequence, many cancer patients have a history of cardiovascular disease and are treated with antiplatelet or anticoagulant drugs. When additional treatment with a TKI is started, the clinical problem of an enhanced bleeding risk may arise. Another treatment issue is that some oral anticoagulants and TKIs (e.g. ibrutinib) have a common metabolic pathway (cytochrome P450 3A4), which can affect the pharmacokinetics of either drug. These interactions between TKIs and antithrombotic treatments need to be further investigated.

Conclusions

Several of the TKIs used for the treatment of cancer can increase the risk of bleeding. For some, but not all, of these, the bleeding tendency is linked to a lowering of platelet count and/or an impairment of platelet function. On the basis of their binding profiles, some other TKIs are predicted to have an antiplatelet effect, although no bleeding side effects have been reported so far. For a third group of TKIs, no published data on platelets are available. Given the suspected role of platelets in tumor progression, platelet inhibition may be a clinically relevant side effect of several TKIs. Together, the available knowledge shows that there must be awareness of the potential antiplatelet effects of TKIs, which are likely to be further enhanced in combination with antithrombotic drugs.

Addendum

B. M. E. Tullemans reviewed the literature, compiled tables and Fig. 1, and wrote the manuscript. J. W. M. Heemskerk was responsible for the outline, and wrote and revised the manuscript. M. J. E. Kuijpers was responsible for the outline, reviewed the literature, and wrote and revised the manuscript.

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Disclosure of Conflict of Interests

M. Kuijpers and J. W. M. Heemskerk report receiving grants from Pfizer during the conduct of the study. B. M. E. Tullemans states that she has no conflict of interest.

References

1 Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. *Physiol Rev* 2013; **93**: 327–58.
2 Morowski M, Vogtle T, Kraft P, Kleinschnitz C, Stoll G, Nieswandt B. Only severe thrombocytopenia results in bleeding and defective thrombus formation in mice. *Blood* 2013; **121**: 4938–47.
3 Baaten CCFMJ, Ten Cate H, van der Meijden PEJ, Heemskerk JWM. Platelet populations and priming in hematological diseases. *Blood Rev* 2017; **31**: 389–99.
4 Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. *Blood* 2015; **126**: 582–8.
5 Cooke NM, Egan K, McFadden S, Grogan L, Breathnach OS, O'Leary J, Hennessy BT, Kenny D. Increased platelet reactivity in patients with late-stage metastatic cancer. *Cancer Med* 2013; **2**: 564–70.
6 Sabrkhany S, Kuijpers MJE, van Kuijk SMJ, Sanders L, Pineda S, Olde Damink SWM, Dingemans AC, Griffioen AW, Oude Egbrink MGA. A combination of platelet features allows detection of early-stage cancer. *Eur J Cancer* 2017; **80**: 5–13.
7 Best MG, Sol N, Kooi I, Tannous J, Westerman BA, Rustenburg F, Schellen P, Verschueren H, Post E, Koster J, Ylstra B, Ameziane N, Dorsman J, Smit EF, Verheul HM, Noske DP, Reijneveld JC, Nilsson RJA, Tannous BA, Wesseling P, *et al.* RNA-seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell* 2015; **28**: 666–76.
8 Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, Honn KV. Platelets and cancer: a casual or causal relationship: revisited. *Cancer Metastasis Rev* 2014; **33**: 231–69.
9 Lin RJ, Afshar-Kharghan V, Schafer AI. Paraneoplastic thrombocytosis: the secrets of tumor self-promotion. *Blood* 2014; **124**: 184–7.
10 Goubran HA, Burnouf T, Radosevic M, El-Ekiaby M. The platelet–cancer loop. *Eur J Intern Med* 2013; **24**: 393–400.
11 Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; **6**: 389–95.

12 Klement GL, Yip TT, Cassiola F, Kikuchi L, Cervi D, Podust V, Italiano JE, Wheatley E, Abou-Slaybi A, Bender E, Almog N, Kieran MW, Folkman J. Platelets actively sequester angiogenesis regulators. *Blood* 2009; **113**: 2835–42.
13 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–74.
14 Gross S, Rahal R, Stransky N, Lengauer C, Hoeflich KP. Targeting cancer with kinase inhibitors. *J Clin Invest* 2015; **125**: 1780–9.
15 Swieringa F, Kuijpers MJ, Heemskerk JW, van der Meijden PE. Targeting platelet receptor function in thrombus formation: the risk of bleeding. *Blood Rev* 2014; **28**: 9–21.
16 Levade M, Severin S, Gratacap MP, Ysebaert L, Payrastre B. Targeting kinases in cancer therapies: adverse effects on blood platelets. *Curr Pharm Des* 2016; **22**: 2315–22.
17 Wu P, Nielsen TE, Clausen MH. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol Sci* 2015; **36**: 422–39.
18 Fabian MA, Biggs WH 3rd, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias JM, Mehta SA, *et al*. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* 2005; **23**: 329–36.
19 Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhart DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM, *et al*. A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* 2008; **26**: 127–32.
20 Yu H, Steeghs N, Nijenhuis CM, Schellens JH, Beijnen JH, Huitema AD. Practical guidelines for therapeutic drug monitoring of anticancer tyrosine kinase inhibitors: focus on the pharmacokinetic targets. *Clin Pharmacokinet* 2014; **53**: 305–25.
21 Gotink KJ, Verheul HM. Anti-angiogenic tyrosine kinase inhibitors: what is their mechanism of action? *Angiogenesis* 2010; **13**: 1–14.
22 Hartmann JT, Haap M, Kopp HG, Lipp HP. Tyrosine kinase inhibitors – a review on pharmacology, metabolism and side effects. *Curr Drug Metab* 2009; **10**: 470–81.
23 Eskens FA, Verweij J. The clinical toxicity profile of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR) targeting angiogenesis inhibitors; a review. *Eur J Cancer* 2006; **42**: 3127–39.
24 Moslehi JJ, Deininger M. Tyrosine kinase inhibitor-associated cardiovascular toxicity in chronic myeloid leukemia. *J Clin Oncol* 2015; **33**: 4210–18.
25 Bair SM, Choueiri TK, Moslehi J. Cardiovascular complications associated with novel angiogenesis inhibitors: emerging evidence and evolving perspectives. *Trends Cardiovasc Med* 2013; **23**: 104–13.
26 Choueiri TK, Schutz FA, Je Y, Rosenberg JE, Bellmunt J. Risk of arterial thromboembolic events with sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. *J Clin Oncol* 2010; **28**: 2280–5.
27 Nieswandt B, Watson SP. Platelet–collagen interaction: is GPVI the central receptor? *Blood* 2003; **102**: 449–61.
28 Senis YA, Mazharian A, Mori J. Src family kinases: at the forefront of platelet activation. *Blood* 2014; **124**: 2013–24.
29 Watson SP, Auger JM, McCarty OJ, Pearce AC. GPVI and integrin alphaIIb beta3 signaling in platelets. *J Thromb Haemost* 2005; **3**: 1752–62.
30 Navarro-Nunez L, Langan SA, Nash GB, Watson SP. The physiological and pathophysiological roles of platelet CLEC-2. *Thromb Haemost* 2013; **109**: 991–8.
31 Shattil SJ, Newman PJ. Integrins: dynamic scaffolds for adhesion and signaling in platelets. *Blood* 2004; **104**: 1606–15.
32 Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood* 2012; **120**: e73–82.
33 Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, Hocker M, Treiber DK, Zarrinkar PP. Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol* 2011; **29**: 1046–51.
34 Shatzel JJ, Olson SR, Tao DL, McCarty OJT, Danilov AV, DeLoughery TG. Ibrutinib-associated bleeding: pathogenesis, management and risk reduction strategies. *J Thromb Haemost* 2017; **15**: 835–47.
35 Molina-Cerrillo J, Alonso-Gordoa T, Gajate P, Grande E. Bruton's tyrosine kinase (BTK) as a promising target in solid tumors. *Cancer Treat Rev* 2017; **58**: 41–50.
36 Rauf F, Festa F, Park JG, Magee M, Eaton S, Rinaldi C, Betanzos CM, Gonzalez-Malerva L, LaBaer J. Ibrutinib inhibition of ERBB4 reduces cell growth in a WNT5A-dependent manner. *Oncogene* 2018; **37**: 2237–50.
37 Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, Grant B, Sharman JP, Coleman M, Wierda WG, Jones JA, Zhao W, Heerema NA, Johnson AJ, Sukbuntherng J, Chang BY, Clow F, Hedrick E, Buggy JJ, James DF, *et al*. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2013; **369**: 32–42.
38 Caron F, Leong DP, Hillis C, Fraser G, Siegal D. Current understanding of bleeding with ibrutinib use: a systematic review and meta-analysis. *Blood Adv* 2017; **1**: 772–8.
39 Alberelli MA, Innocenti I, Sica S, Laurenti L, De Candia E. PO-54 – clinical and laboratory characterization of platelet dysfunction caused by ibrutinib treatment in patients with chronic lymphocytic leukemia. *Thromb Res* 2016; **140**(Suppl. 1): S196.
40 Bye AP, Unsworth AJ, Vaiyapuri S, Stainer AR, Fry MJ, Gibbins JM. Ibrutinib inhibits platelet integrin alphaIIbbeta3 outside-in signaling and thrombus stability but not adhesion to collagen. *Arterioscler Thromb Vasc Biol* 2015; **35**: 2326–35.
41 Kamel S, Horton L, Ysebaert L, Levade M, Burbury K, Tan S, Cole-Sinclair M, Reynolds J, Filshie R, Schischka S, Khot A, Sandhu S, Keating MJ, Nandurkar H, Tam CS. Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation. *Leukemia* 2015; **29**: 783–7.
42 Levade M, David E, Garcia C, Laurent PA, Cadot S, Michallet AS, Bordet JC, Tam C, Sie P, Ysebaert L, Payrastre B. Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions. *Blood* 2014; **124**: 3991–5.
43 Lipsky AH, Farooqui MZ, Tian X, Martyr S, Cullinane AM, Nghiem K, Sun C, Valdez J, Niemann CU, Herman SE, Saba N, Soto S, Marti G, Uzel G, Holland SM, Lozier JN, Wiestner A. Incidence and risk factors of bleeding-related adverse events in patients with chronic lymphocytic leukemia treated with ibrutinib. *Haematologica* 2015; **100**: 1571–8.
44 Rigg RA, Aslan JE, Healy LD, Wallisch M, Thierheimer ML, Loren CP, Pang J, Hinds MT, Gruber A, McCarty OJ. Oral administration of Bruton's tyrosine kinase inhibitors impairs GPVI-mediated platelet function. *Am J Physiol Cell Physiol* 2016; **310**: C373–80.
45 Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, Chaves J, Wierda WG, Awan FT, Brown JR, Hillmen P, Stephens DM, Ghia P, Barrientos JC, Pagel JM, Woyach J, Johnson D, Huang J, Wang X, Kaptein A, *et al*. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2016; **374**: 323–32.
46 Bye AP, Unsworth AJ, Desborough MJ, Hildyard CAT, Appleby N, Bruce D, Kriek N, Nock SH, Sage T, Hughes CE, Gibbins JM. Severe platelet dysfunction in NHL patients receiving ibrutinib is absent in patients receiving acalabrutinib. *Blood Adv* 2017; **1**: 2610–23.

47 Yang K, Fu LW. Mechanisms of resistance to BCR-ABL TKIs and the therapeutic strategies: a review. *Crit Rev Oncol Hematol* 2015; **93**: 277–92.
48 Breccia M, Stefanizzi C, Cannella L, Latagliata R, Frustaci AM, Carmosino I, Santopietro M, Alimena G. Differences in hematological and non-hematological toxicity during treatment with imatinib in patients with early and late chronic phase chronic myeloid leukemia. *Leuk Lymphoma* 2008; **49**: 2328–32.
49 Kostos L, Burbury K, Srivastava G, Prince HM. Gastrointestinal bleeding in a chronic myeloid leukaemia patient precipitated by dasatinib-induced platelet dysfunction: case report. *Platelets* 2015; **26**: 809–11.
50 Mustafa Ali MK, Sabha MM, Al-Rabi KH. Spontaneous subdural hematoma in a patient with Philadelphia chromosome-positive acute lymphoblastic leukemia with normal platelet count after dasatinib treatment. *Platelets* 2015; **26**: 491–4.
51 Gratacap MP, Martin V, Valera MC, Allart S, Garcia C, Sie P, Recher C, Payrastre B. The new tyrosine-kinase inhibitor and anticancer drug dasatinib reversibly affects platelet activation in vitro and in vivo. *Blood* 2009; **114**: 1884–92.
52 Quintas-Cardama A, Han X, Kantarjian H, Cortes J. Tyrosine kinase inhibitor-induced platelet dysfunction in patients with chronic myeloid leukemia. *Blood* 2009; **114**: 261–3.
53 Li R, Grosser T, Diamond SL. Microfluidic whole blood testing of platelet response to pharmacological agents. *Platelets* 2017; **28**: 457–62.
54 Shah NP, Kantarjian HM, Kim DW, Rea D, Dorlhiac-Llacer PE, Milone JH, Vela-Ojeda J, Silver RT, Khoury HJ, Charbonnier A, Khoroshko N, Paquette RL, Deininger M, Collins RH, Otero I, Hughes T, Bleickardt E, Strauss L, Francis S, Hochhaus A. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. *J Clin Oncol* 2008; **26**: 3204–12.
55 Mazharian A, Ghevaert C, Zhang L, Massberg S, Watson SP. Dasatinib enhances megakaryocyte differentiation but inhibits platelet formation. *Blood* 2011; **117**: 5198–206.
56 Alhawiti N, Burbury KL, Kwa FA, O'Malley CJ, Shuttleworth P, Alzard M, Hamadi A, Grigg AP, Jackson DE. The tyrosine kinase inhibitor, nilotinib potentiates a prothrombotic state. *Thromb Res* 2016; **145**: 54–64.
57 Loren CP, Aslan JE, Rigg RA, Nowak MS, Healy LD, Gruber A, Druker BJ, McCarty OJ. The BCR-ABL inhibitor ponatinib inhibits platelet immunoreceptor tyrosine-based activation motif (ITAM) signaling, platelet activation and aggregate formation under shear. *Thromb Res* 2015; **135**: 155–60.
58 Neelakantan P, Marin D, Laffan M, Goldman J, Apperley J, Milojkovic D. Platelet dysfunction associated with ponatinib, a new pan BCR-ABL inhibitor with efficacy for chronic myeloid leukemia resistant to multiple tyrosine kinase inhibitor therapy. *Haematologica* 2012; **97**: 1444.
59 Pasvolsky O, Leader A, Iakobishvili Z, Wasserstrum Y, Kornowski R, Raanani P. Tyrosine kinase inhibitor associated vascular toxicity in chronic myeloid leukemia. *Cardiooncology* 2015; **1**: 5–14.
60 Je Y, Schutz FAB, Choueiri TK. Risk of bleeding with vascular endothelial growth factor receptor tyrosine-kinase inhibitors sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. *Lancet Oncol* 2009; **10**: 967–74.
61 Sabrkhany S, Griffioen AW, Pineda S, Sanders L, Mattheij N, van Geffen JP, Aarts MJ, Heemskerk JW, Oude Egbrink MG, Kuijpers MJ. Sunitinib uptake inhibits platelet function in cancer patients. *Eur J Cancer* 2016; **66**: 47–54.
62 Hamilton Z, Lee HJ, Jimenez J, Lane BR, Wang S, Beksac AT, Gillis K, Alagh A, Tobert C, Randall JM, Kane CJ, Millard F, Campbell SC, Derweesh IH. Change in platelet count as a prognostic indicator for response to primary tyrosine kinase inhibitor therapy in metastatic renal cell carcinoma. *BJU Int* 2016; **118**: 927–34.
63 Crist M, Hansen E, Chablani L, Guancial E. Examining the bleeding incidences associated with targeted therapies used in metastatic renal cell carcinoma. *Crit Rev Oncol Hematol* 2017; **120**: 151–62.
64 Keating GM. Afatinib: a review of its use in the treatment of advanced non-small cell lung cancer. *Drugs* 2014; **74**: 207–21.
65 Cao H, Bhuyan AAM, Umbach AT, Bissinger R, Gawaz M, Lang F. Inhibitory effect of afatinib on platelet activation and apoptosis. *Cell Physiol Biochem* 2017; **43**: 2264–76.
66 Cardoso HJ, Figueira MI, Socorro S. The stem cell factor (SCF)/c-KIT signalling in testis and prostate cancer. *J Cell Commun Signal* 2017; **11**: 297–307.
67 Hitchcock IS, Kaushansky K. Thrombopoietin from beginning to end. *Br J Haematol* 2014; **165**: 259–68.
68 Quintas-Cardama A, Verstovsek S. Spleen deflation and beyond: the pros and cons of Janus kinase 2 inhibitor therapy for patients with myeloproliferative neoplasms. *Cancer* 2012; **118**: 870–7.
69 Moore SF, Hunter RW, Harper MT, Savage JS, Siddiq S, Westbury SK, Poole AW, Mumford AD, Hers I. Dysfunction of the PI3 kinase/Rap1/integrin alpha(IIb)beta(3) pathway underlies ex vivo platelet hypoactivity in essential thrombocythemia. *Blood* 2013; **121**: 1209–19.
70 Lu WJ, Lin KC, Huang SY, Thomas PA, Wu YH, Wu HC, Lin KH, Sheu JR. Role of a Janus kinase 2-dependent signaling pathway in platelet activation. *Thromb Res* 2014; **133**: 1088–96.
71 Andrews RK, Arthur JF, Gardiner EE. Targeting GPVI as a novel antithrombotic strategy. *J Blood Med* 2014; **5**: 59–68.
72 Etulain J, Fondevila C, Negrotto S, Schattner M. Platelet-mediated angiogenesis is independent of VEGF and fully inhibited by aspirin. *Br J Pharmacol* 2013; **170**: 255–65.
73 Mezouar S, Darbousset R, Dignat-George F, Panicot-Dubois L, Dubois C. Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo. *Int J Cancer* 2015; **136**: 462–75.
74 Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012; **379**: 1591–601.
75 Mitrugno A, Sylman JL, Ngo AT, Pang J, Sears RC, Williams CD, McCarty OJ. Aspirin therapy reduces the ability of platelets to promote colon and pancreatic cancer cell proliferation: implications for the oncoprotein c-MYC. *Am J Physiol Cell Physiol* 2017; **312**: C176–89.
76 Verheul HM, Hoekman K, Luykx-de Bakker S, Eekman CA, Folman CC, Broxterman HJ, Pinedo HM. Platelet: transporter of vascular endothelial growth factor. *Clin Cancer Res* 1997; **3**: 2187–90.
77 Peterson JE, Zurakowski D, Italiano JE Jr, Michel LV, Connors S, Oenick M, D'Amato RJ, Klement GL, Folkman J. VEGF, PF4 and PDGF are elevated in platelets of colorectal cancer patients. *Angiogenesis* 2012; **15**: 265–73.
78 Verheul HM, Lolkema MP, Qian DZ, Hilkes YH, Liapi E, Akkerman JW, Pili R, Voest EE. Platelets take up the monoclonal antibody bevacizumab. *Clin Cancer Res* 2007; **13**: 5341–7.
79 Williams M, Liu ZW, Woolf D, Hargreaves S, Michalarea V, Menashy R, Kooner I, Wilson E. Change in platelet levels during radiotherapy with concurrent and adjuvant temozolomide for the treatment of glioblastoma: a novel prognostic factor for survival. *J Cancer Res Clin Oncol* 2012; **138**: 1683–8.
80 Chen F, Maridakis V, O'Neill EA, Beals C, Radziszewski W, de Lepeleire I, Van Dyck K, Depre M, Bolognese JA, de Hoon

J, Jacquemin M. A randomized clinical trial comparing point-of-care platelet function assays and bleeding time in healthy subjects treated with aspirin or clopidogrel. *Platelets* 2012; **23**: 249–58.

81 Nagy M, Mastenbroek TG, Mattheij NJA, de Witt S, Clemetson KJ, Kirschner J, Schulz AS, Vraetz T, Speckmann C, Braun A, Cosemans J, Zieger B, Heemskerk JWM. Variable impairment of platelet functions in patients with severe, genetically linked immune deficiencies. *Haematologica* 2018; **103**: 540–9.

82 Carrier M, Le Gal G, Wells PS, Fergusson D, Ramsay T, Rodger MA. Systematic review: the Trousseau syndrome revisited: should we screen extensively for cancer in patients with venous thromboembolism? *Ann Intern Med* 2008; **149**: 323–33.

83 Li J, Zhao X, Chen L, Guo H, Lv F, Jia K, Yv K, Wang F, Li C, Qian J, Zheng C, Zuo Y. Safety and pharmacokinetics of novel selective vascular endothelial growth factor receptor-2 inhibitor YN968D1 in patients with advanced malignancies. *BMC Cancer* 2010; **10**: 529–36.

84 Keller G, Schafhausen P, Brummendorf TH. Bosutinib: a dual SRC/ABL kinase inhibitor for the treatment of chronic myeloid leukemia. *Expert Rev Hematol* 2009; **2**: 489–97.

85 Johnson PJ, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Avina J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, *et al*. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517–24.

86 Choueiri TK, Escudier B, Powles T, Mainwaring PN, Rini BI, Donskov F, Hammers H, Hutson TE, Lee J-L, Peltola K, Roth BJ, Bjarnason GA, Géczi L, Keam B, Maroto P, Heng DYC, Schmidinger M, Kantoff PW, Borgman-Hagey A, Hessel C, *et al*. Cabozantinib versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015; **373**: 1814–23.

87 Langenberg MH, van Herpen CM, De Bono J, Schellens JH, Unger C, Hoekman K, Blum HE, Fiedler W, Drevs J, Le Maulf F, Fielding A, Robertson J, Voest EE. Effective strategies for management of hypertension after vascular endothelial growth factor signaling inhibition therapy: results from a phase II randomized, factorial, double-blind study of Cediranib in patients with advanced solid tumors. *J Clin Oncol* 2009; **27**: 6152–9.

88 Soria J-C, Tan DSW, Chiari R, Wu Y-L, Paz-Ares L, Wolf J, Geater SL, Orlov S, Cortinovis D, Yu C-J, Hochmair M, Cortot AB, Tsai C-M, Moro-Sibilot D, Campelo RG, McCulloch T, Sen P, Dugan M, Pantano S, Branle F, *et al*. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet* 2017; **389**: 917–29.

89 Boespflug A, Thomas L. Cobimetinib and vemurafenib for the treatment of melanoma. *Expert Opin Pharmacother* 2016; **17**: 1005–11.

90 Shaw AT, Yasothan U, Kirkpatrick P. Crizotinib. *Nat Rev Drug Discov* 2011; **10**: 897–8.

91 Gibney GT, Zager JS. Clinical development of dabrafenib in BRAF mutant melanoma and other malignancies. *Expert Opin Drug Metab Toxicol* 2013; **9**: 893–9.

92 Reckamp KL, Giaccone G, Camidge DR, Gadgeel SM, Khuri FR, Engelman JA, Koczywas M, Rajan A, Campbell AK, Gernhardt D, Ruiz-Garcia A, Letrent S, Liang J, Taylor I, O'Connell JP, Janne PA. A phase 2 trial of dacomitinib (PF-00299804), an oral, irreversible pan-HER (human epidermal growth factor receptor) inhibitor, in patients with advanced non-small cell lung cancer after failure of prior chemotherapy and erlotinib. *Cancer* 2014; **120**: 1145–54.

93 Aguilera DG, Tsimberidou AM. Dasatinib in chronic myeloid leukemia: a review. *Ther Clin Risk Manag* 2009; **5**: 281–9.

94 Joensuu H, Blay JY, Comandone A, Martin-Broto J, Fumagalli E, Grignani G, Del Muro XG, Adenis A, Valverde C, Pousa AL, Bouche O, Italiano A, Bauer S, Barone C, Weiss C, Crippa S, Camozzi M, Castellana R, LeCesne A. Dovitinib in patients with gastrointestinal stromal tumour refractory and/or intolerant to imatinib. *Br J Cancer* 2017; **117**: 1278–85.

95 Sharman J, Hawkins M, Kolibaba K, Boxer M, Klein L, Wu M, Hu J, Abella S, Yasenchak C. An open-label phase 2 trial of entospletinib (GS-9973), a selective spleen tyrosine kinase inhibitor, in chronic lymphocytic leukemia. *Blood* 2015; **125**: 2336–43.

96 Rocha-Lima CM, Raez LE. Erlotinib (Tarceva) for the treatment of non-small-cell lung cancer and pancreatic cancer. *Pharm. Therap* 2009; **34**: 554–64.

97 McAdoo SP, Tam FWK. Fostamatinib disodium. *Drugs Future* 2011; **36**: 273–82.

98 Shi Y, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, Wang D, Li Q, Qin S, Hu C, Zhang Y, Chen J, Cheng Y, Feng J, Zhang H, Song Y, Wu YL, Xu N, Zhou J, Luo R, *et al*. Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol* 2013; **14**: 953–61.

99 Blay J-Y, von Mehren M. Nilotinib: a novel, selective tyrosine kinase inhibitor. *Semin Oncol* 2011; **38**: S3–9.

100 Kim S-B, Dent R, Im S-A, Espié M, Blau S, Tan AR, Isakoff SJ, Oliveira M, Saura C, Wongchenko MJ, Kapp AV, Chan WY, Singel SM, Maslyar DJ, Baselga J. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol* 2017; **18**: 1360–72.

101 Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J. Randomized study of lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol* 2010; **28**: 1124–30.

102 Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, Habra MA, Newbold K, Shah MH, Hoff AO, Gianoukakis AG, Kiyota N, Taylor MH, Kim SB, Krzyzanowska MK, Dutcus CE, de las Heras B, Zhu J, Sherman SI. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. *N Engl J Med* 2015; **372**: 621–30.

103 Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, Kim DS, Kolb M, Nicholson AG, Noble PW, Selman M, Taniguchi H, Brun M, Le Maulf F, Girard M, Stowasser S, *et al*. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014; **370**: 2071–82.

104 Goss G, Tsai C-M, Shepherd FA, Bazhenova L, Lee JS, Chang G-C, Crino L, Satouchi M, Chu Q, Hida T, Han J-Y, Juan O, Dunphy F, Nishio M, Kang J-H, Majem M, Mann H, Cantarini M, Ghiorghiu S, Mitsudomi T. Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. *Lancet Oncol* 2016; **17**: 1643–52.

105 Jain P, Kantarjian H, Jabbour E, Gonzalez GN, Borthakur G, Pemmaraju N, Daver N, Gachimova E, Ferrajoli A, Kornblau S, Ravandi F, O'Brien S, Cortes J. Ponatinib as first-line treatment for patients with chronic myeloid leukaemia in chronic phase: a phase 2 study. *Lancet Haematol* 2015; **2**: e376–83.

106 Ferraro D, Zalcberg J. Regorafenib in gastrointestinal stromal tumors: clinical evidence and place in therapy. *Ther Adv Med Oncol* 2014; **6**: 222–8.

107 Casaluce F, Sgambato A, Maione P, Sacco PC, Santabarbara G, Gridelli C. Selumetinib for the treatment of non-small cell lung cancer. *Exp Opin Invest Drugs* 2017; **26**: 973–84.

108 Lugowska I, Koseła-Paterczyk H, Kozak K, Rutkowski P. Trametinib: a MEK inhibitor for management of metastatic melanoma. *Onco Targets Ther* 2015; **8**: 2251–9.
109 Wells SA, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol* 2012; **30**: 134–41.
110 Hecht JR, Trarbach T, Hainsworth JD, Major P, Jager E, Wolff RA, Lloyd-Salvant K, Bodoky G, Pendergrass K, Berg W, Chen BL, Jalava T, Meinhardt G, Laurent D, Lebwohl D, Kerr D. Randomized, placebo-controlled, phase III study of first-line oxaliplatin-based chemotherapy plus PTK787/ZK 222584, an oral vascular endothelial growth factor receptor inhibitor, in patients with metastatic colorectal adenocarcinoma. *J Clin Oncol* 2011; **29**: 1997–2003.
111 Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, Hirth P. Vemurafenib: the first drug approved for BRAF-mutant cancer. *Nat Rev Drug Discov* 2012; **11**: 873–86.